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NOTES ON THE ACTIVITY OF EARTHWORMS

II. Observations on diapause in the earthworm *A. caliginosa*

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Introduction

In summer, in the field quite a number of earthworms are found coiled up in small rounded chambers, which are often plastered with faeces. The worms are pink, more transparent than normal, have an empty alimentary canal and are not easily roused to activity.

The tendency to go into this 'diapause' differs with the species. From the middle of May, up to the middle or end of September, hardly any active individuals of *Allolobophora longa* are to be found, whereas in most cases some active specimens of *A. caliginosa* can be found. It is a useful observation of Michon (5) that earthworms with red pigment never go into diapause; only species without red pigment do so. So far we did not meet with exceptions.

Stephenson (7) in discussing the sexual activity of earthworms writes: 'I think general experience in this country (England, *ed.*) agrees with Miss Pickford (6) that sexual worms may be found at all seasons, though they are on the whole much less common in winter than in summer.

Avel (2, 3) and Abeloos and Avel (1) state that in *A. terrestris (= longa)* and *A. caliginosa* the active portion of the sexual cycles takes place in winter, and that the worms are quiescent sexually during the warmer months from April onwards (locality not mentioned). This 'diapause' is not due to the desiccation of the soil, or to a rise of temperature, since it occurred in worms kept in a constant environment; it is not merely a state of rest after the sexual phase, since immature specimens, and castrated worms, undergo a similar period of inactivity. The gut is emptied, the worms retire deeply into the soil, and coil up within a cyst; the appetite for food ceases. The diapause is due to internal factors; it ends at the time of the autumn rains, and the worms then rouse and eat ravenously. It would seem that the modification of the usual cycle described by these authors is an adaptation to the climatic conditions of the particular region.'

The observations by Avel and Abeloos et Avel on diapause are very much to the point; it is therefore a step back if Michon (5) contributes diapause to drought only.

We do agree with most of the observations of Avel and Abeloos; however, that diapause should be due to internal factors (only) has not been proved. The fact that Stephenson at the time overlooked the importance of diapause in earthworms could be an indication that this phenomenon is less common in England than it is on the continent. This could equally well point to direct climatic influences and not necessarily to an adaptation of the worms to the different climates.

Since during our experiments with earthworms we always were confronted with this phenomenon of diapause which interfered in a most unpleasant way with our experiments, an attempt was made to find a way to overcome these difficulties. The use of

red-pigmented earthworms which cannot go into diapause is not a solution of the problem, as these animals die under the circumstances in which the other ones go into diapause.

Methods

A series of smaller experiments was carried out, in which specimens of *Allolobophora caliginosa* were kept in small breeding cells ('flats') as described by DOEKSEN (4). As a standard, a good sandy garden soil was used, after thorough mixing and sieving. The pH (water) of this soil was brought to 7.0 by the addition of $CaCO_3$. To study the influence of surface activity of the soil, 10% clay, nearly free from organic matter and 10% activated charcoal respectively were mixed with the standard soil.

Differences in pH were brought about by the addition of the necessary quantity of $CaCO_3$ or H_2SO_4. After thorough mixing, the soil was kept in the laboratory for some weeks, mixed again and the pH determined. We are well aware that, by these treatments, the different soils were not fully comparable, but at the time there were no better means to reach the pH-differences needed.

In all experiments, ten flats with two worms each were used per treatment. They were kept at constant temperatures; the moisture content was high and almost constant. Care was taken that demineralized water was always present in the boxes in which series of 10 flats were kept.

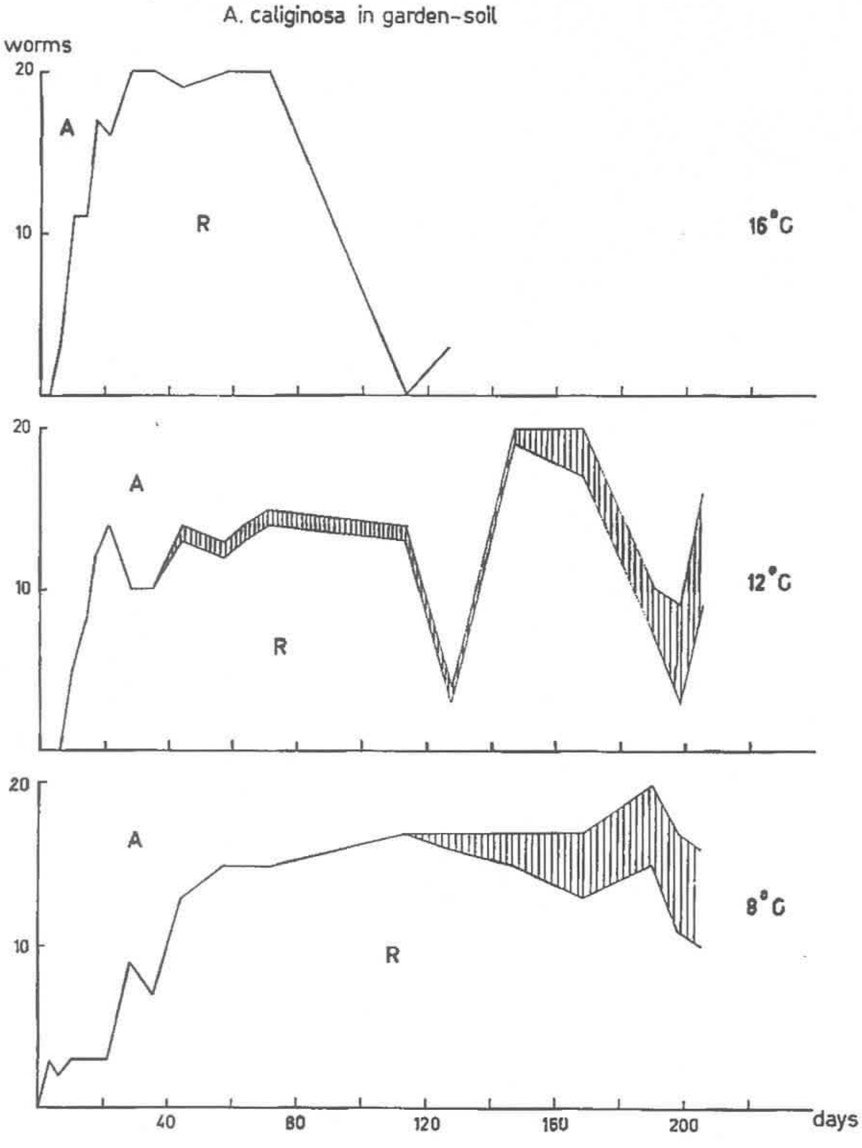


FIG. 1. The effect of temperature on *A. caliginosa* in a sandy garden soil. A: active worms. R: worms resting; Shading: dead worms.

In earlier experiments diapause was obtained under all conditions, but since STEPHENSON (7) suggested that differences in climate might be the reason for the supposed different behaviour of earthworms in England and in France, it seemed necessary to study the influence of temperature on diapause. We chose three temperatures, which are not abnormal for soil conditions, *viz.* 8°, 12° and 16°C.

As from the start diapause was suspected to be the result of some kind of poisoning, it was thought likely that the soil, expecially its buffering capacity, might have influence on the phenomenon. Clay and charcoal were therefore added to the soil as described.

In all, there were three 'soils' at three temperatures. The data from this experiment are brought together in the figures 1–3 incl.

Unfortunately, after 112 days, the 16°C-incubator broke down, the temperature for some time rising to over 30°C which of course proved lethal to the worms.

In the diagrams the lower part, marked R, represents the worms that are in diapause; the upper part A are the worms that are active, while shading indicates the dead specimens.

It is evident, that with rising temperature, diapause is induced quicker and is more general; this holds for the three soils. During periods in which only part of the worms

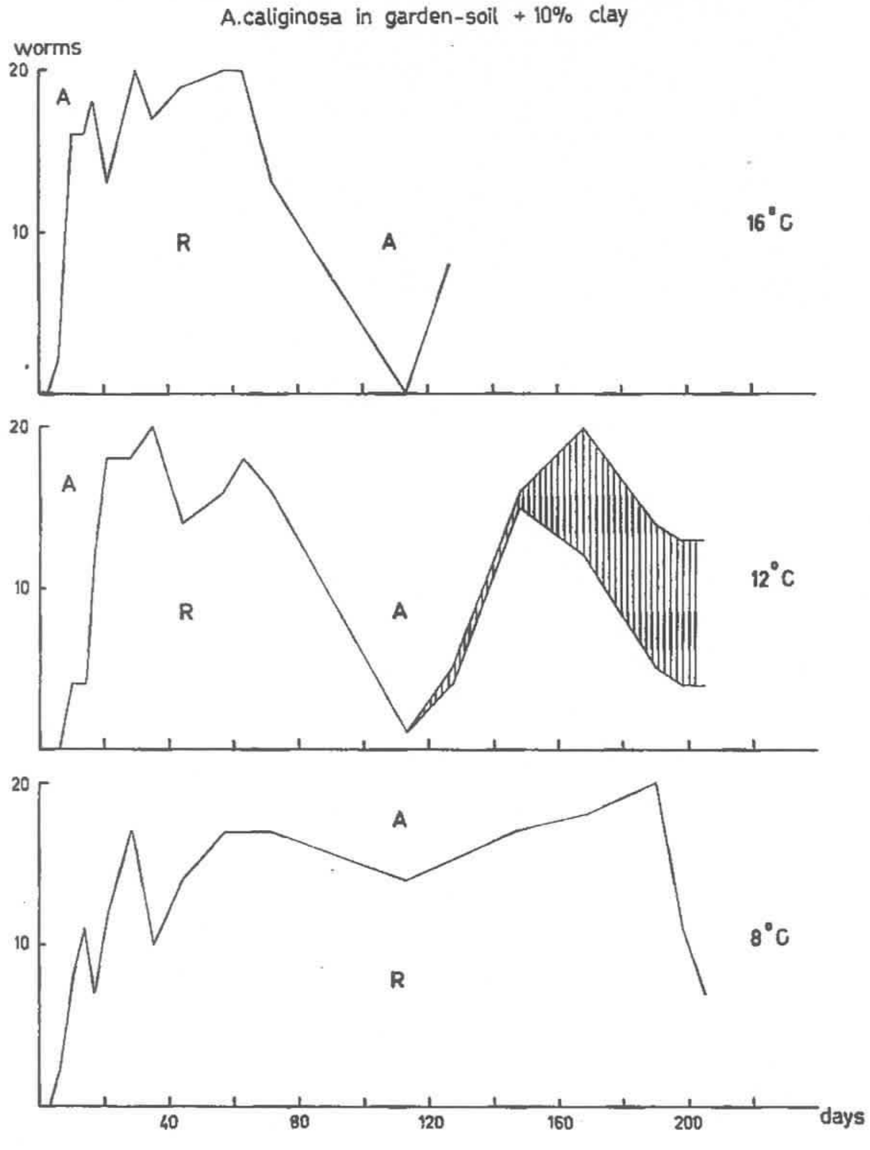


FIG. 2. The effect of temperature on *A. caliginosa* in a sandy garden soil + 10% clay. Explanations see fig. 1.

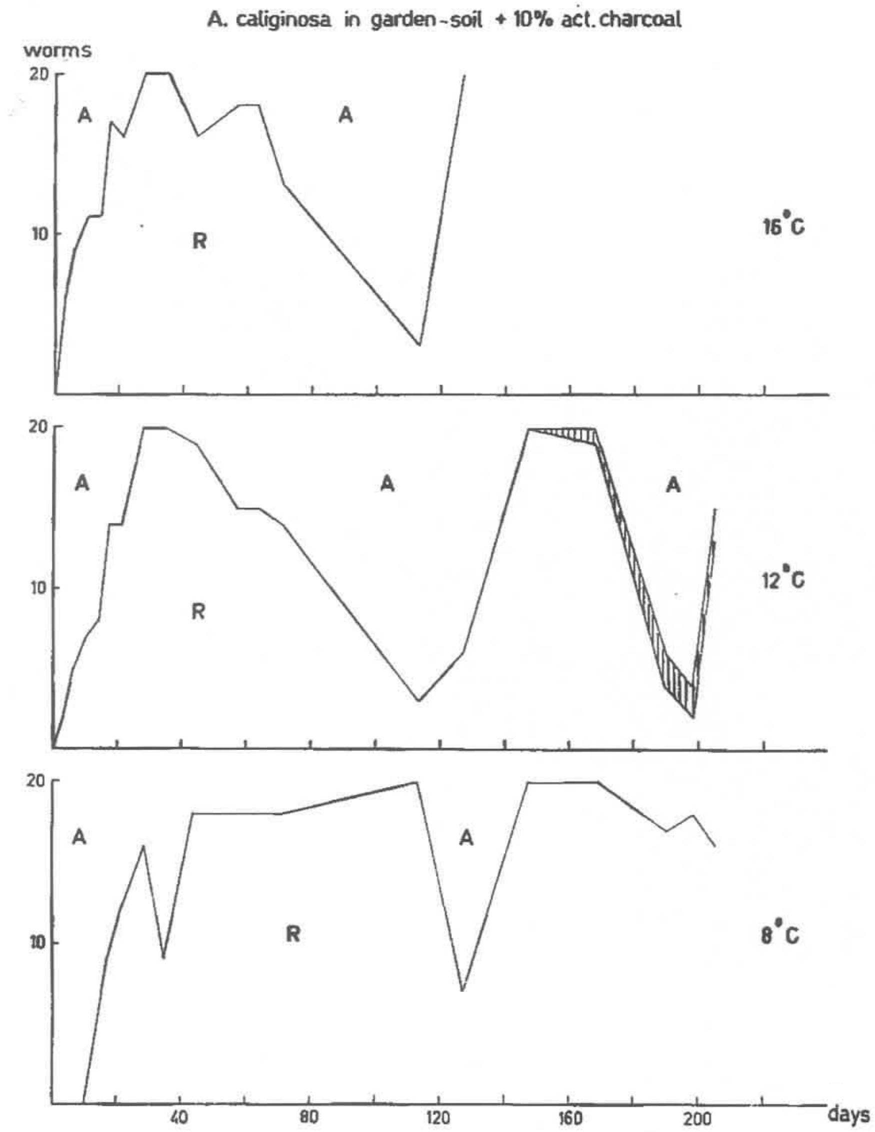


FIG. 3. The effect of temperature on *A. caliginosa* in a sandy garden soil + 10% activated charcoal. Explanations see fig. 1.

are in diapause, not always the same individuals are resting the whole time, but they may be active again while others go into diapause. This explains the small peaks in the diagrams.

It is interesting to note, that after a period in which a high percentage of the animals is at rest, almost general activity occurs, followed by a new period of diapause which is shorter than the first one, followed again by a period of activity.

This alternation of activity and rest is only slightly indicated at 8°C.

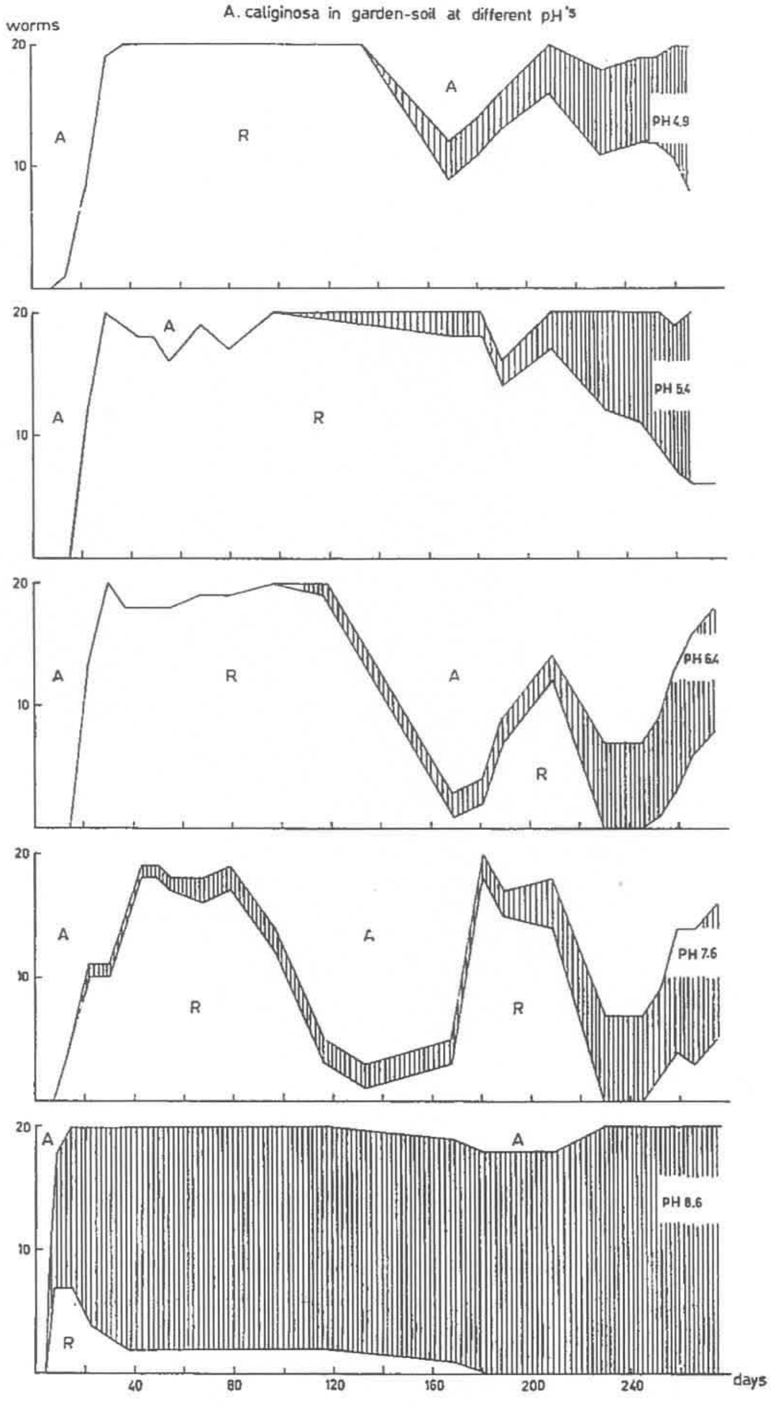
There is an indication that clay and charcoal shorten the time of diapause.

INFLUENCE OF THE pH OF THE SOIL ON DIAPAUSE

There is a general opinion that earthworms are sensitive to soils with a low pH-value or soils poor in calcium. As diapause often appears to be a way for the animal to escape and survive unfavourable conditions, it is indicated to study the influence of as many of these conditions as may be thought of. Therefore, five different pH-levels were chosen of nearly the same soil as described before, to study their effect on diapause, at 16°C; fig. 4 gives the result in diagrams.

It is evident that pH 8.6 is far too high for *A. caliginosa*. There is high mortality, and the living individuals go into diapause very soon, but most of them die after some weeks.

FIG. 4. The influence of pH of a sandy garden soil on diapause and death rate in *A. caliginosa*. Explanations see fig. 1.



The diagrams of pH 7.6 and 6.4 show the characteristics of those of fig. 1–3 incl., *viz.* an alternation of activity and diapause with a tendency of the following periods of diapause being shorter.

At pH 6.4 the first period of diapause is very long and intensive, but followed by a well-pronounced period of activity; at pH 5.4 and 4.9 there only is a faint indication of greater activity as is the case at lower temperatures.

DISCUSSION

From the data, given in the figures1–4 incl. it is clear, that diapause is induced neither by abnormal temperatures, by changes in temperature nor by drought, as the moderate soil temperatures were kept constant and free water was always present in the flats. Often worms were lying coiled up, partly immersed in water.

It is neither likely that an internal rhythm should make the animals go into diapause, as all worms caught in the field at different times of the year behave in the same way.

Since in the field diapause is restricted to summer but is induced neither by temperature nor by drought, there must be conditions in our experimental procedure, which in the field are restricted to summer.

There are two possibilities: 1. The absence of water percolation, a washing out of the soil, both in the field in summer and in our flats. 2. The limited quantity of soil with which the worms come into contact. In the field, high temperatures and drought must influence worm activity adversely, which greatly restricts the amount of soil with which the animals come into contact, as is always the case in our flats, independent of temperature and moisture.

At this stage of our investigation we therefore assume that diapause must be induced by some substance which is primarily excreted by the worms themselves and may be changed by micro-organisms. The same or other micro-organisms are able to break it down under suitable conditions.

This would explain the changes from activity to diapause vice versa: During the active period a relatively great quantity of the supposed material is formed. Its concentration increases till the worms go into diapause after which the excretion decreases. Micro-organisms, which from the start must have been breaking down the material, now are able to lower its concentration to a level at which the worms can be active again. As the concentration of the active agent depends on the amount of soil with which it is diluted, to restrict the movements of the worms must result in locally high concentrations.

Presumably, the active agent is not easily broken down, but it is likely that the micro-organisms that are able to do so, will increase their numbers, so that a second period of diapause will have to be shorter than the first one.

As there is no great difference in the activity of earthworms at 8 °C or 12 °C, but as at 8 °C hardly any re-activation of the worms occurs, it must be assumed, that the micro-organisms involved are more strongly influenced by temperature than the earthworms and that at lower temperatures they are not able to break down the supposed material fast enough to bring diapause to an end.

The influence of pH on the duration of diapause could equally well be an influence on the activity of micro-organisms, especially bacteria.

Adsorbents as clay and activated charcoal could partly inactivate the material which could explain the effect of these products on diapause.

Acting on the assumptions discussed here, we made further experiments on which will be reported in due course.

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